ENDOCRINE DISRUPTORS: IMPACT ON NORTH CAROLINA FISHERIES
December 1, 2008

The Issue

There is growing concern (as evidenced by numerous news media reports) about increasing levels of pharmaceuticals and personal care products in aquatic environments. Many of these chemicals, in very small concentrations, function as endocrine disruptors, which interfere with natural biological processes of various aquatic animals. The health of North Carolina's coastal fisheries depends upon optimal biological processes to support natural populations – and, we do not know whether we have a problem.

Recent research, including a few studies in North Carolina, reveals the impact of selected endocrine disruptors on crustaceans and mollusks (**Table 1**). At concentrations as low as 0.05 ppb, a spectrum of chemicals has been shown to negatively affect the reproduction and physiology (e.g., filtration, molting, etc.) of test animals.

The flow of many streams contains municipal wastewater treatment facility effluents. Mixing large volumes of wastewater upstream of drinking water intakes and estuarine environments can expose humans and aquatic organisms to a variety of carcinogenic and endocrine disrupting chemicals. This issue has become a growing concern as identified by the news media and resource managers.

The Situation

We do not know if we have concentrations of endocrine disruptors in our coastal waters. But, we have not looked! There are some suspicious declines in populations of some species in certain areas, but a lack of monitoring and research makes it impossible to evaluate. We do know, however, that various endocrine disruptors come from sewage, pesticides, antibiotics and human-health pharmaceuticals. Many pharmaceuticals ingested by humans are excreted in urine and enter surface waters through wastewater effluent. Unused drugs are often flushed down toilets, and pesticides and other chemicals enter surface water through runoff.

The U.S. Geological Survey conducts a nationwide monitoring program looking for a large variety of chemicals in selected sampling stations (**Table 2**). In North Carolina, the USGS conducted a limited amount of monitoring for endocrine disrupting chemicals in freshwater reaches of the Tar, Neuse, and Cape Fear river basins. Prescription drugs (antibiotics and other medications), non-prescription drugs, flame retardants, plasticizers, fragrances, pesticides, detergent metabolites, antimicrobial agents, and other suspected endocrine disruptors have been detected. North Carolina's estuaries were not targeted in these studies.

North Carolina needs to develop a program to investigate and evaluate the potential for endocrine disruptors to affect our fisheries, which would include:

- Estuarine monitoring of the concentration and prevalence of priority chemicals of concern
- Specific, critical research on the effects of chemicals on fishery species, particularly blue crab, oysters, and fish
- Education and outreach
- A plan for safe disposal of pharmaceuticals, pesticides and antibiotics
- A plan for removal of chemicals from wastewater and run-off

Monitoring Program

In order to determine the potential for impact of endocrine disruptors, we need a site-specific, compound specific monitoring program. The Neuse River and its estuary could serve as a model and there is an existing water quality sampling grid. The N.C. Division of Water Quality could expand its sampling program to include collection of water samples for analyses. Analyses could be accomplished at USGS laboratories already equipped to measure endocrine disruptors. Monitoring could also be expanded by including water samples collected when sampling for oyster pathogens occurrence, bacterial sampling for shellfish sanitation, fisheries larval and juvenile sampling, etc.

It is important that critical chemicals be selected for analyses, rather than going to the expense of trying to analyze everything. Chemicals like fipronil (frontline), bisphenol A (certain plastics), alpha and beta estrodiol (hormones), antibiotic degradation products, alachlor and other high-use pesticides, juvenile hormone analogs (mosquito control), etc. should be prime candidates. A monitoring task force comprised of USGS, DWQ, DMF, NCDA, Shellfish Sanitation, as well as representative researchers, could identify a list of most likely problematic chemicals.

Research Program

Invertebrates are unique in that their endocrine systems control not only sexual development and reproduction, but also growth, molting, limb regeneration, diapause, metamorphosis, pigmentation, immune function and the production of pheromones. For the most part, these aspects of endocrine regulation, while they may be susceptible to the effects of environmental contaminants, have not been fully examined and their influence on population structure and population stability is unknown. While limited research has identified some potential impacts, it is critical that highly focused research be developed for critical species.

The blue crab is North Carolina's most valuable fishery, but is recently in decline. Water quality degradation is one of several important factors that contribute to the decline of blue crab populations. Therefore, we need to focus research on the response of blue crabs to various endocrine disruptors present in North Carolina's waters. Developing larvae and juveniles are vulnerable and should be tested, as well as the potential effects on molting. Juvenile hormones used in some insecticides and their impact on development should be investigated. Research needs to be expanded to evaluate impacts at the population level.

North Carolina's native oysters are an important fishery and the state is currently investing substantial resources for restoration and hatchery development. It is important to determine the effects of endocrine disruptors on reproduction, larval development and reef formation. Research results must be translated to the population level.

Research focused on risk assessment is necessary to determine potential impact of endocrine disruptors on the well-being of North Carolina's fisheries.

Education and Outreach Activities

The seriousness of potential adverse impacts from endocrine disruptors argues for outreach initiatives now. While we wait for monitoring and research to identify specific concerns, we can initiate education and outreach efforts to generate fundamental public understanding of potential impacts. Several initiatives could include:

- Brochure emphasizing potential detrimental impacts on fisheries could be distributed to schools, museums, fairs, extension programs or via enclosure in utility bills.

- Encourage more judicious use of chemical agents at home and at work.

- Promote awareness of existing waste management and recycling programs, including development of new or revised approaches to waste management.

- Involve the media in communicating information from research and monitoring, as well as public concern.

The EPA 319 continues to provide support for research, workshops and development of educational materials. We should continue to take advantage of federal support. Also, Cooperative Extension, Sea Grant, Industrial Extension, N.C. Department of Agriculture, and other outreach programs, should be encouraged to address the issue.

The Pesticide Disposal Assistance Program already serves as an important collection and disposal mechanism for unused pesticides. This program could be expanded to include safe and private disposal of pharmaceuticals.

Effective Removal from Waste Streams

Conventional wastewater treatment facilities are not suited for effective removal of most of the chemicals listed here. To upgrade and/or modify existing wastewater systems to be effective at removal would be prohibitively expensive and would be difficult to sell to the taxpayer. Recently developed information indicates that engineered wetlands provide a very cost-effective solution. However, additional research, demonstration and evaluation are needed to quantify the roles of absorption, biotransformation and photolysis in such systems, as well as cost analyses. Moreover,

work needs to be done to plan for dealing with tons of biomass produced by an engineered wetland.

Recommendations

1. Establish a monitoring program to determine the presence and concentrations of selected chemicals in the Neuse River and its estuary. Organize a monitoring Task Force made of USGS, DWQ, DMF, NCDA, Shellfish Sanitation and selected researchers to determine sites and target chemicals.

2. Provide support for research on blue crabs and oysters and their response to endocrine disruptors at critical life stages. Encourage the Blue Crab and Shellfish Research and Fisheries Resource Grant programs, EPA, Sea Grant, Water Resources Research Institute, and other agencies to make research on endocrine disruptors a priority.

3. Develop brochure(s) emphasizing the role of endocrine disruptors on North Carolina fisheries for use in schools, museums, fairs, media, etc.

4. Promote awareness of existing waste management and recycling programs, including development of new or revised approaches to include pharmaceuticals.

5. Expand the N.C. Pesticide Disposal Assistance Program to include unused and outdated pharmaceuticals.

Table 1: Results of research on effects (from Robert Roer and Pat McClellan-Green).

CRUSTACEAN ENDOCRINE DISRUPTERS				
Class	**Compounds**	**Conc.'s**	**Effects**	**Organisms**
Human hormones and hormone-like substances	17ß-estradiol, bisphenol A, 17α-ethinylestradiol, *p*-octylphenol, tamoxifen, 4-(tert)-octylphenol, 4-n-nonylphenol, androstenedione, diethyl phthalate, diethylstilbestrol, PCB29 (2,4,5-trichlorobiphenyl), pyrene	As low as 0.1 ppb	Toxicity, inc. in female/male sex ratio, intersex males, molting/molt enzyme abnormalities, abnormal larval development, altered egg production & maturation	Amphipods, copepods, water fleas, shrimp, crabs
Insecticides, nematocides & fungicides (non-hormonal)	Araclor 1242, dieldrin, Heptachlor, Lindane, endosulfan, enamectin benzoate, fenarimol, agricultural run-off	As low as 0.05 ppb	Molting/molt enzyme abnormalities, inc. in male/female sex ratio, intersex males, toxicity	Water fleas, crabs, lobsters
Juvenile hormone insecticides (act to interrupt the insect life cycle)	Fenoxycarb, methoprene, pyriproxifen	As low as 0.1 ppb	Inc. in male/female sex ratio, abnormal/extended larval development, molting/molt enzyme abnormalities, delay in reproduction, dec. fecundity, all female broods	Water fleas, mysids, shrimp, crabs
MOLLUSK ENDOCRINE DISRUPTERS				
Human hormones and hormone-like substances (incl. pharmaceuticals)	17b-Estradiol 17a-Ethynylestradiol 4-Nonylphenol Testosterone Fluoxetine (Prozac)	As low as 0.05 ppb	Decreased reproduction, increased vitellogenisis, sperm abnormalities	Oysters, clams, scallops
Insecticides, nematocides and fungicides (non-hormonal)	Lindane Endosulfan PCB, Araclor	As low as 0.5 ppb	Reduced filtration, gonad atrophy, decreased reproduction	Oysters, snails, clams, scallops
Organics and metals	Benzopryene Organotins	As low as 0.1 ppb	Delay sexual maturity decreased reproduction, abnormal development	Oysters

Table 2: List of compounds in the USGS monitoring program. **Bold text** indicates known or suspected endocrine disruptors.

WASTEWATER INDICATORS		
Analyte	**Reporting Level**	**Unit**
Cotinine	0.4	µg/L
5-Methyl-1H-benzotriazole	0.08	µg/L
Anthraquinone	0.16	µg/L
Acetophenone	0.4	µg/L
Acetyl hexamethyl tetrahydronaphthalene (AHTN)	0.5	µg/L
Anthracene	0.08	µg/L
1,4-Dichlorobenzene	0.08	µg/L
Benzo[a]pyrene	0.12	µg/L
Benzophenone	0.12	µg/L
Bromacil	0.4	µg/L
Bromoform	0.08	µg/L
3-tert-Butyl-4-hydroxy anisole (BHA)	0.6	µg/L
Caffeine	0.1	µg/L
Caffeine-C13 (surrogate)		pct
Camphor	0.1	µg/L
Carbaryl	1	µg/L
Carbazole	0.08	µg/L
Chlorpyrifos	0.12	µg/L
Cholesterol	1.4	µg/L
3-beta-Coprostanol	1	µg/L
Isopropylbenzene	0.1	µg/L
Fluoranthene-d10 (surrogate)		pct
Bisphenol A-d3 (surrogate)		pct
Decafluorobiphenyl (surrogate)		pct
N,N-diethyl-meta-toluamide (DEET)	0.1	µg/L
Diazinon	0.08	µg/L
Bisphenol A	0.4	µg/L
Triethyl citrate (ethyl citrate)	0.2	µg/L
Tetrachloroethylene	0.08	µg/L
Fluoranthene	0.08	µg/L
Hexahydrohexamethylcyclopentabenzopyran (HHCB)	0.5	µg/L
Indole	0.14	µg/L
Isoborneol	0.06	µg/L
Isophorone	0.08	µg/L
Isoquinoline	0.2	µg/L
d-Limonene	0.04	µg/L
Menthol	0.2	µg/L
Metalaxyl	0.08	µg/L
Metolachlor	0.08	µg/L
Naphthalene	0.1	µg/L

WASTEWATER INDICATORS *CONTINUED*		
Analyte	**Reporting Level**	**Unit**
1-Methylnaphthalene	0.1	µg/L
2,6-Dimethylnaphthalene	0.12	µg/L
2-Methylnaphthalene	0.08	µg/L
4-Nonylphenol diethoxylate, (sum of all isomers) aka NP2EO	5	µg/L
4-Octylphenol diethoxylate, aka OP2EO	1	µg/L
4-Octylphenol monoethoxylate, aka OP1EO	1	µg/L
p-Cresol	0.18	µg/L
4-Cumylphenol	0.1	µg/L
para-Nonylphenol (total) (branched)	1	µg/L
4-n-Octylphenol	0.16	µg/L
4-tert-Octylphenol	1	µg/L
Phenanthrene	0.08	µg/L
Phenol	0.2	µg/L
Pentachlorophenol	2	µg/L
Tributyl phosphate	0.2	µg/L
Triphenyl phosphate	0.1	µg/L
Tris(2-butoxyethyl)phosphate	0.4	µg/L
Tris(2-chloroethyl)phosphate	0.1	µg/L
Prometon	0.18	µg/L
Pyrene	0.08	µg/L
Methyl salicylate	0.1	µg/L
3-Methyl-1(H)-indole (Skatole)	0.08	µg/L
beta-Sitosterol	1.6	µg/L
beta-Stigmastanol	1.2	µg/L
Triclosan	0.2	µg/L
Tris(dichlorisopropyl)phosphate	0.12	µg/L

HORMONES		
Analyte	**Reporting Level**	**Unit**
11-Ketotestosterone	0.8	ng/L
17-alpha-Estradiol	0.8	ng/L
17-alpha-Ethynylestradiol-2,4,16,16-d4 (surrogate)		pct
17-beta-Estradiol	0.8	ng/L
17-beta-Estradiol-d4 (surrogate)		pct
17-alpha-Ethynylestradiol	0.8	ng/L
Norethindrone	0.8	ng/L
Norethindrone-2,2,4,6,6,10-d6 (surrogate)		pct
4-Androstene-3,17-dione	2	ng/L
4-Androstene-3,17-dione-2,2,4,6,6,16,16-d7 (surrogate)		pct
cis-Androsterone	0.8	ng/L
Cholesterol	4000	ng/L
Cholesterol-d7 (surrogate)		pct
3-beta-Coprostanol	4000	ng/L
Dihydrotestosterone	0.8	ng/L
Dihydrotestosterone-1,2,4,5a-d4 (surrogate)		pct
Epitestosterone	4	ng/L
Equilenin	2	ng/L
Equilin	4	ng/L
Estriol	0.8	ng/L
Estriol-2,4,17-d3 (surrogate)		pct
Estrone	0.8	ng/L
Estrone-2,4,16,16-d4 (surrogate)		pct
Mestranol	0.8	ng/L
Mestranol-2,4,16,16-d4 (surrogate)		pct
Progesterone	4	ng/L
Progesterone-2,2,4,6,6,17a,21,21,21-d9 (surrogate)		pct
trans-Diethylstilbestrol	0.8	ng/L
Testosterone	0.8	ng/L
Testosterone-2,2,4,6,6-d5 (surrogate)		pct
trans-Diethyl-1,1,1',1'-d4-stilbesterol-3,3',5,5'-d4 (surrogate)		pct

ANTIBIOTICS (*degradation product)		
Analyte	**Reporting Level**	**Unit**
FLUOROQUINOLINES		
Ciprofloxacin	0.005	µg/L
Enrofloxacin	0.005	µg/L
Lomefloxacin	0.005	µg/L
Norfloxacin	0.005	µg/L
Ofloxacin	0.005	µg/L
Sarafloxacin	0.005	µg/L
MACROLIDES		
Azithromycin	0.005	µg/L
Erythromycin	0.008	µg/L
*Erythromycin-H2O	0.008	µg/L
Roxithromycin	0.005	µg/L
Tylosin	0.005	µg/L
Virginiamycin	0.005	µg/L
SULFONAMIDES		
Sulfachloropyridazine	0.005	µg/L
Sulfadiazine	0.100	µg/L
Sulfadimethoxine	0.005	µg/L
Sulfamethoxazole	0.005	µg/L
Sulfamethazine	0.005	µg/L
Suflathiazole	0.020	µg/L
TETRACYCLINES		
Chlorotetracyline	0.010	µg/L
Oxytetracycline	0.010	µg/L
Tetracycline	0.010	µg/L
Doxycycline	0.010	µg/L
*Epi-chlorotetracycline	0.010	µg/L
*Epi-iso-chlorotetracycline	0.010	µg/L
*Epi-oxytetracycline	0.010	µg/L
*Epi-tetracycline	0.010	µg/L
*Iso-chlorotetracycline	0.010	µg/L
OTHERS		
Chloramphenicol	0.100	µg/L
Lincomycin	0.005	µg/L
Ormetoprim	0.005	µg/L
Trimethoprim	0.005	µg/L
PHARMACEUTICALS		
Carbamazepine	0.005	µg/L
Ibuprofen	0.050	µg/L

HUMAN-HEALTH PHARMACEUTICALS		
Analyte	**Reporting Level**	**Unit**
1,7-Dimethylxanthine (p-Xanthine)	0.1	ug/L
Acetaminophen	0.08	ug/L
Albuterol (Salbutamol)	0.04	ug/L
Caffeine	0.06	ug/L
Carbamazepine	0.04	ug/L
Carbamazepine-d10 (surrogate)		pct
Codeine	0.04	ug/L
Cotinine	0.026	ug/L
Dehydronifedipine	0.06	ug/L
Diltiazem	0.04	ug/L
Diphenhydramine	0.05	ug/L
Ethyl nicotinate-d4 (surrogate)		pct
Sulfamethoxazole	0.1	ug/L
Thiabendazole	0.1	ug/L
Trimethoprim	0.04	ug/L
Warfarin	0.06	ug/L

PESTICIDES		
Analyte	**Reporting Level**	**Unit**
1-Naphthol	0.04	ug/L
2,4,5-T (surrogate)		pct
2,4-D	0.02	ug/L
2,4-D methyl ester	0.04	ug/L
2,4-D plus 2,4-D methyl ester	0.02	ug/L
2,4-DB	0.02	ug/L
2,6-Diethylaniline	0.006	ug/L
2-Chloro-2,6-diethylacetanilide	0.01	ug/L
2-Chloro-4-isopropylamino-6-amino-s-triazine {CIAT}	0.06	ug/L
2-Chloro-4-isopropylamino-6-amino-s-triazine {CIAT}	0.014	ug/L
2-Chloro-6-ethylamino-4-amino-s-triazine {CEAT}	0.08	ug/L
2-Ethyl-6-methylaniline	0.01	ug/L
2-Hydroxy-4-isopropylamino-6-ethylamino-s-triazine {OIET}	0.04	ug/L
3(4-Chlorophenyl)-1-methyl urea	0.12	ug/L
3,4-Dichloroaniline	0.006	ug/L
3,5-Dichloroaniline	0.008	ug/L
3-Hydroxycarbofuran	0.04	ug/L
4-Chloro-2-methylphenol	0.005	ug/L
Acetochlor	0.006	ug/L
Acifluorfen	0.04	ug/L
Alachlor	0.006	ug/L
Aldicarb	0.12	ug/L
Aldicarb sulfone	0.08	ug/L
Aldicarb sulfoxide	0.06	ug/L
alpha-Endosulfan	0.006	ug/L
alpha-HCH-d6 (surrogate)		pct
Atrazine	0.007	ug/L
Azinphos-methyl	0.12	ug/L
Azinphos-methyl-oxon	0.042	ug/L
Azoxystrobin	0.008	ug/L
Barban (surrogate)		pct
Bendiocarb	0.04	ug/L
Benfluralin	0.01	ug/L
Benomyl	0.04	ug/L
Bensulfuron-methyl	0.06	ug/L
Bentazon	0.04	ug/L
Boscalid	0.008	ug/L
Bromacil	0.02	ug/L
Bromoxynil	0.12	ug/L
Caffeine	0.06	ug/L
Caffeine-C13 (surrogate)		pct
Carbaryl	0.04	ug/L

PESTICIDES *CONTINUED*		
Analyte	**Reporting Level**	**Unit**
Carbofuran	0.02	ug/L
Chloramben, methyl ester	0.1	ug/L
Chlorimuron-ethyl	0.08	ug/L
Chlorothalonil	0.008	ug/L
Chlorpyrifos	0.005	ug/L
Chlorpyrifos, oxygen analog	0.0562	ug/L
cis-Permethrin	0.01	ug/L
cis-Propiconazole	0.006	ug/L
Clopyralid	0.06	ug/L
Cyanazine	0.02	ug/L
Cycloate	0.02	ug/L
Cyfluthrin	0.016	ug/L
Cypermethrin	0.014	ug/L
Cyproconazole	0.008	ug/L
Dacthal	0.003	ug/L
Dacthal monoacid	0.02	ug/L
Desulfinylfipronil	0.012	ug/L
Desulfinylfipronil amide	0.029	ug/L
Diazinon	0.005	ug/L
Diazinon, oxygen analog	0.006	ug/L
Diazinon-d10		pct
Dicamba	0.04	ug/L
Dichlorprop	0.02	ug/L
Dichlorvos	0.013	ug/L
Dicrotophos	0.0843	ug/L
Dieldrin	0.009	ug/L
Dimethoate	0.006	ug/L
Dinoseb	0.04	ug/L
Diphenamid	0.04	ug/L
Disulfoton	0.04	ug/L
Disulfoton sulfone	0.014	ug/L
Diuron	0.04	ug/L
Endosulfan sulfate	0.022	ug/L
EPTC	0.002	ug/L
Ethion	0.006	ug/L
Ethion monoxon	0.021	ug/L
Ethoprophos	0.012	ug/L
Fenamiphos	0.029	ug/L
Fenamiphos sulfone	0.053	ug/L
Fenamiphos sulfoxide	0.2	ug/L
Fenuron	0.04	ug/L
Fipronil	0.02	ug/L
Fipronil sulfide	0.013	ug/L

PESTICIDES *CONTINUED*		
Analyte	**Reporting Level**	**Unit**
Fipronil sulfone	0.024	ug/L
Flumetsulam	0.06	ug/L
Fluometuron	0.04	ug/L
Fonofos	0.01	ug/L
Hexazinone	0.008	ug/L
Imazaquin	0.04	ug/L
Imazethapyr	0.04	ug/L
Imidacloprid	0.06	ug/L
Iprodione	0.01	ug/L
Isofenphos	0.006	ug/L
lambda-Cyhalothrin	0.004	ug/L
Linuron	0.02	ug/L
Malaoxon	0.02	ug/L
Malathion	0.016	ug/L
MCPA	0.06	ug/L
MCPB	0.06	ug/L
Metalaxyl	0.0069	ug/L
Metconazole	0.008	ug/L
Methidathion	0.004	ug/L
Methiocarb	0.04	ug/L
Methomyl	0.12	ug/L
Metolachlor	0.01	ug/L
Metribuzin	0.012	ug/L
Metsulfuron methyl	0.14	ug/L
Molinate	0.003	ug/L
Myclobutanil	0.008	ug/L
Neburon	0.02	ug/L
Nicosulfuron	0.1	ug/L
Norflurazon	0.02	ug/L
Oryzalin	0.04	ug/L
Oxamyl	0.12	ug/L
Oxyfluorfen	0.006	ug/L
p,p'-DDD	0.0012	ug/L
p,p'-DDE	0.0019	ug/L
p,p'-DDT	0.0014	ug/L
Parathion-methyl	0.008	ug/L
Pendimethalin	0.012	ug/L
Phorate	0.04	ug/L
Phorate oxygen analog	0.027	ug/L
Phosmet	0.0079	ug/L
Phosmet oxon	0.0511	ug/L
Picloram	0.12	ug/L
Polychlorinated biphenyls, total	0.1	ug/L

PESTICIDES *CONTINUED*		
Analyte	**Reporting Level**	**Unit**
Prometon	0.01	ug/L
Prometryn	0.0059	ug/L
Propanil	0.006	ug/L
Propargite	0.04	ug/L
Propham	0.04	ug/L
Propiconazole	0.04	ug/L
Propoxur	0.04	ug/L
Propyzamide	0.004	ug/L
Pyraclostrobin	0.008	ug/L
Siduron	0.02	ug/L
Simazine	0.006	ug/L
Sulfometuron-methyl	0.06	ug/L
Tebuconazole	0.008	ug/L
Tebuthiuron	0.016	ug/L
Tefluthrin	0.0033	ug/L
Terbacil	0.04	ug/L
Terbufos	0.018	ug/L
Terbufos oxygen analog sulfone	0.045	ug/L
Terbuthylazine	0.0083	ug/L
Tetrachlorophthalonitrile		pct
Tetraconazole	0.008	ug/L
Thiobencarb	0.01	ug/L
trans-Propiconazole	0.008	ug/L
Tribufos	0.035	ug/L
Triclopyr	0.08	ug/L
Trifloxystrobin	0.008	ug/L
Trifluralin	0.009	ug/L